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LIVER NECROSIS AND VENOUS THROMBOSIS IN HORSES
ACTIVELY IMMUNIZED WITH DIPHTHERIA AND
TETANUS TOXINS AND WITH STREPTO-
COCCI AND THEIR PRODUCTS.*†

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OUR search of the literature dealing with the anatomical and histological findings in man or animals dead of diphtheria, tetanus, or streptococcus infections, either natural or artificial, has failed to reveal to us definite records of the conditions similar to those which we are about to describe.‡

Law in his extensive work on *veterinary medicine* gives no descriptions of liver necrosis in the horse exactly comparable to the condition in our series. However, from the statements in his chapter on "Hepatic Hemorrhage and Rupture" we gather the impression that various degenerations of liver cells and subsequent traumatic hemorrhage and rupture of the capsule are far more common in the horse than in man. From his pathological and clinical descriptions the usual cases of hemorrhage and rupture are undoubtedly much milder than those in our horses, as the latter rarely live over 10 minutes after the onset of symptoms, while he gives five hours to five days as the period of duration.

Klitine¹ calls attention to fatty degeneration of the liver cells of guinea-pigs, the subjects of chronic experimental diphtheria. Dzierzowsky² also mentions the occurrence of fatty degeneration of the livers of horses which have been used in diphtheria antitoxin production.

* Read at the Sixth Annual Meeting of the American Association of Pathologists and Bacteriologists held at Baltimore, May 18, 1906.

† Received for publication June 4, 1906.

‡ That liver necroses have been frequently found in horses, the subjects of diphtheria antitoxin production, is no secret to many of those in charge of laboratories where such work is carried on, but no description of this condition has been published so far as we have been able to learn.

¹ *Arch. d. Sci. Biol.* (St. Petersburg), 1900, 8, p. 2.

² *Ibid.*, 1902, 9, p. 293.

Pettit and Girard¹ found complete necrosis of the spleen in a horse used in the production of antiplague serum.

We have to report the finding at autopsy of more or less diffuse necrosis of the liver, or thrombosis of some of the branches of the portal or splenic veins or of the pulmonary artery, either alone or in combination, in 12 horses. These animals had been subjected to repeated subcutaneous injections of the artificially produced toxins of the diphtheria or the tetanus bacillus, or of the various preparations of living or dead cultures, or of suspensions of dysentery bacilli or streptococci. In this series there are seven horses treated with diphtheria toxin, two with tetanus, two with streptococcus, and one with dysentery. A horse treated with diphtheria toxin and killed because of its crippled condition is added to the series for purposes of comparison, as its organs were found to be normal. (See Table 1.)

Superficial postmortem examinations were made of the first three diphtheria horses, and incomplete autopsies, in that no special search for thrombosis was made, were performed on the second three horses in the series. (See Table 1.)

TABLE 1.
SHOWING AUTOPSY FINDINGS RELATING TO NECROSIS AND THROMBOSIS.*

HORSE	SERVICE				LIVER		THROMBOSIS OF VEINS		
	Disease	Duration Yrs. Mos.	Sudden Death	Killed	Necrosis	Rupture	Liver	Spleen	Pulmo- nary Artery
11.....	Diphtheria	1 11	+		Diffuse	+	?	?	?
17.....	"	2 1	+		"	+	?	?	?
28.....	"	2	+		"	+	?	?	?
36.....	Dysentery	11	+		"	+	?	?	?
37.....	Streptococcus	1 9	+		"	+	?	?	?
38.....	"	1 9½	+		"	+	?	?	?
21.....	Tetanus	3 3	+		"	+	+	+	—
29.....	"	3 7	+		"	+	+	—	+
59.....	Diphtheria	8		+†	—	—	—	—	—
57.....	"	4		+†	—	—	—	—	+
43.....	"	2 2	+		Diffuse	+	+	+	—
50.....	"	1 7	+		Localized	—	—	—	—
56.....	"	1 5		+†	—	—	—	—	—

As we have not been fortunate enough to be present when any of the horses died from the effects of hemorrhage and rupture of the

¹ *Compt. rend. de la Soc. de Biol.*, 1905, 58, p. 272.

* The horses reported upon in this series include all those which have died, or have been killed, after a year and a half of service under the various treatments, since the observations were started.

† Horses 59 and 57 were killed because they were in such a disturbed state that it was impossible to control them. Horse 56 was hardly able to walk on account of the nearly immovable condition of the joints of its legs, and as its serum was of no value it was killed as a matter of economy. Its tissues were normal.

liver, we are unable to give a detailed clinical picture of the symptoms preceding death in these cases. The general conditions as described by an attendant are as follows: The animals are apparently in their usual condition. They may have recently eaten a normal meal. Attention is first attracted to them by a rapid noisy breathing. The animal exhibits signs of collapse, and shortly falls to the floor. The breathing becomes very labored and the animal struggles. This stage is followed by a rapid and progressive loss of power, and death ensues. The whole course may not last over 10 minutes.

At autopsy the peritoneal cavity contains a very large amount of fluid blood or serum and clots. The liver is usually very soft and



FIG. 1.—Necrosis, Hemorrhage, and Rupture of Liver. Horse 43.

friable, and is handled with difficulty. The capsule presents one or more ruptures, and at such places the liver substance is broken up into stringy fragments, interspersed with blood clots. (See Figure 1.)

Our attention was first called to the occurrence of thrombosis in association with diffuse necrosis of the liver by the findings at the autopsy of Horse 21, which occurred April 9, 1905. As is shown in Table 1, this animal had been in active service for the production of tetanus antitoxin for a period of over three years, and was in apparent excellent physical condition when she died in the manner already described. As the protocol of this animal gives a fairly representative picture of the conditions found in the other horses in the series, it will be given in full.

On opening the abdomen a large amount of bloody fluid escapes and many light red clots. The intestines are distended. The glands of the mesentery in the ileocolic

region are enlarged and soft. The liver for the most part presents a yellowish-gray color; within the right lobe an encapsulated hemorrhagic mass. On section it is found that the entire liver is necrotic, presenting a grayish color and a consistence resembling that of thick oatmeal. In some places there is added to this the yellowish tinge of bile. The hemorrhage in the right lobe is found to be subcapsular and infiltrates the liver substance. It is essentially a hematoma beneath the capsule. No remnant of normal liver can be made out. Everywhere with slight pressure the liver substance can be removed leaving only the connective tissue framework.

The portal veins at their entrance to the liver are everywhere filled with smooth, firm, yellowish-white clots but slightly streaked with red. (See Fig. 2.) These clots project into all the smaller branches and are definitely adherent to the vessel wall. In most instances they completely occlude the vessels in which they lie. On section

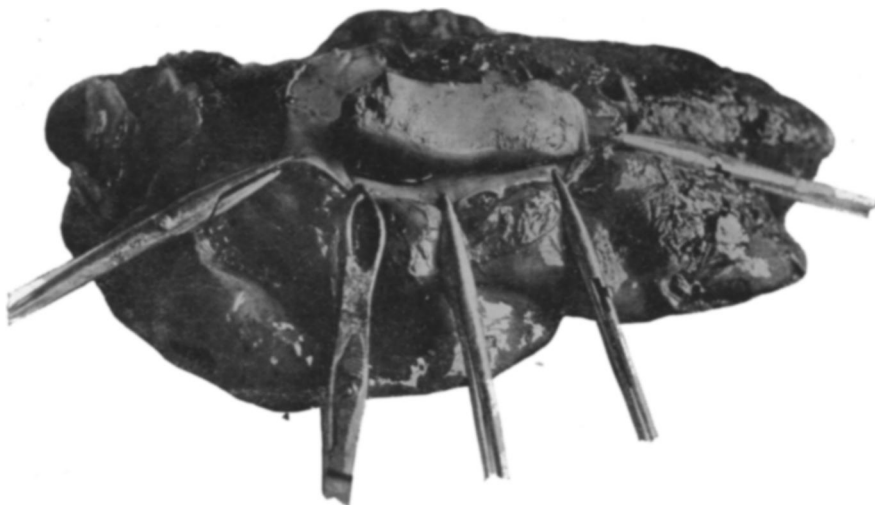


FIG. 2.—Thrombosis in Portal Vein. Horse 21.

through the liver small, firm, yellow, rigid clots protrude from the surface. The hepatic artery is free as are also the aorta and vena cava.

The spleen shows slight thickening of the capsule, and on section are found here and there a few soft swollen areas of a darker red than the surrounding tissue and in the center of each a clot similar to those found in the liver. On dissecting the main branch of the splenic vein an adherent thrombus can be followed for some distance. The splenic artery is free.

There is no accumulation of fluid in either pleural or pericardial cavity. The heart is pale, but otherwise negative. The lungs are pale and neither congested nor edematous. There are no thrombi in the pulmonary, coronary, or other vessels of the thorax. The kidneys are pale and cloudy. The adrenals are swollen and soft.

Careful examination of vascular system shows no thrombi in vessels other than those of liver and spleen.

Anatomical diagnosis.—Widespread necrosis of liver. Hematoma of the liver.

Focal hemorrhages in the spleen. Hemoperitoneum. Thrombosis of the portal and splenic veins.

Histology, liver.—Sections taken at random from different portions of the liver show practically complete necrosis without leucocytic infiltration. Here and there the nuclei in small portions of a lobule may be distinguished, as may also those of the structures in the larger portal spaces, but otherwise the destruction is complete. Much bile pigment is present in the necrotic cells.

Sections passing through the periphery of the hematoma in the right lobe show this mass to be surrounded by a definite fibrous capsule from which prolongations pass into the adjacent necrotic tissue. In this fibrous tissue is a diffuse deposition of old blood pigment.

Other sections show loose granulation tissue penetrating and replacing the blood clot. Here and there in the older portions are newly formed bile ducts and liver cells, the latter being large, pale, and multinucleated.

Sections through five thrombi differing in size show a uniform structure—a definite network of fibrin enmeshing large numbers of leucocytes. At the periphery the fibrin is compact and hyalin, forming broad bands. Small bands of similar hyalin are found also in the other portions. Only an occasional red corpuscle is seen.

Spleen.—Section through the occluded vein shows a thrombus, similar in structure to those found in the liver and intimately adherent to the vessel wall, which is greatly thinned and infiltrated with lymphoid cells. In the tissues about the vessels a diffuse hemorrhage has occurred. Elsewhere the spleen is normal.

Lymph nodes.—The sinuses are dilated and closely packed with large, pale, endothelial cells. These are but slightly phagocytic. In the lymph nodes are many plasma cells with, however, no diminution of the lymphoid cells. Throughout the lymphoid tissue is an abundant deposition of old blood pigment.

Adrenal.—Normal.

Lung.—Congestion, moderate edema in focal areas and slight thickening of walls of alveoli.

Kidney.—Cloudy swelling of epithelium of tubules, few foci of lymphoid cell infiltration.

Heart muscle.—Cloudy swelling.

Histological diagnosis.—Necrosis of liver with repair about hematoma. Mixed thrombi of portal vessels. Thrombosis of splenic veins with perivascular hemorrhage. Endothelial hyperplasia of lymph nodes with deposition of old blood pigment. Congestion and edema of lung. Cloudy swelling of heart and kidney.

ABSTRACTS FROM CLINICAL HISTORIES AND PROTOCOL.

HORSE II.

Clinical notes.—Horse about 10 years old. Used in the diphtheria service. Had stiff leg joints at time of entering, which gradually became worse until horse was almost constantly confined to its stall.

The maximum antitoxin strength of its serum was approximately 500 units per c.c. During the last six months of its life the horse showed progressive emaciation. After a year and 11 months of treatment the horse suddenly developed the usual symptoms of hepatic rupture, and died in a few minutes.

Autopsy notes.—A superficial examination shows diffuse necrosis and hemorrhage of the liver, with rupture of the capsule and consequent hemoperitoneum.

HORSE 17.

Clinical notes.—Sound horse of uncertain age. Diphtheria service. Always remained well and active during period of treatment of two years and one month. Antitoxic strength of serum varied from 200 to 400 units per c.c.

The animal retained its usual weight until death, which occurred suddenly after the development of the usual symptoms of hepatic rupture.

Autopsy notes.—Examination incomplete. Diffuse necrosis of the liver with hemorrhage, rupture, and hemoperitoneum.

HORSE 28.

Clinical notes.—Sound horse about 12 years old. Diphtheria service. Active during entire period of treatment of two years. Maximum antitoxic strength of serum 400 units per c.c. Gradual loss of flesh during last two months of life. Had frequent abscesses at the sites of injection with accompanying high temperatures. Died during the night.

Autopsy notes.—Examination incomplete. Diffuse necrosis of the liver, with hemorrhage, rupture, and hemoperitoneum.

HORSE 36.

Clinical notes.—Sound horse about 10 years of age. Dysentery service. Progressively increasing doses of autolyzed suspensions of surface growth of agar cultures of two strains of *B. dysenteriae* were injected alternately into this horse. They produced very severe reactions, during which the temperature would often rise to 104° F., or over, and the site of the injection would become very much swollen and painful, and the animal would refuse to eat for several days.

After a total of 496 c.c. of these autolyzed suspensions of dysentery bacilli had been administered, living broth cultures of the same strains were administered. They caused much less vigorous systemic reactions.

About seven weeks before death the animal developed a severe diarrhea, which was checked with difficulty. It also began to lose weight, and finally became much emaciated. The animal was found dead early in the morning.

Autopsy notes.—Diffuse necrosis of the liver with hemorrhage, rupture, and hemoperitoneum. Other organs, including intestinal mucous membrane, normal microscopically. Cultures from the usual organs were negative. No thrombosis was noted in any of the vessels of the liver, spleen, or lungs, but attention was not directed particularly toward this point.

Histological diagnosis.—Intense diffuse granular degeneration with irregular focal areas of necrosis in the liver.

HORSE 37.

Clinical notes.—Sound active horse of about 12 years of age. Streptococcus service. Received, in gradually increased doses, a total of about three and a half liters of autolyzed salt solution suspensions of a rabbit-virulent streptococcus, grown on the surface of large agar plates, and later about half a liter of broth cultures of the same streptococcus. Small subcutaneous abscesses followed the later injections. The animal retained its weight and activity during the treatment. It was found dead one morning.

Autopsy notes.—Diffuse necrosis of the liver with hemorrhage, rupture, and hemoperitoneum.

HORSE 38.

Clinical notes.—Streptococcus service. Age uncertain, but animal appeared to be at least 20 years old. Had somewhat crippled legs. Became emaciated during the last two months of life. The treatment was the same as was given Horse 37 and was started at the same time, except that another streptococcus was used. This animal also died during the night, about two weeks after the death of Horse 37, and the autopsy findings were in general the same as those of that animal.

HORSE 21.

Clinical notes.—Sound active mare about 16 years old. Tetanus service. Always well. Remained active and retained its weight during life. She died in the manner already described. (Full protocol will be found in the text.)

HORSE 29.

Clinical notes.—Sound active horse about 10 years of age. Tetanus service. Remained well until about two months before death, when it had frequent rises of temperature and difficulty of breathing which were directly due to the toxin injections. During the last half-year had frequent acute abscesses at sites of injection. This horse died in the manner already described.

Autopsy notes, anatomical diagnosis.—Diffuse necrosis of the liver with subcapsular hemorrhages and rupture. Hemoperitoneum. Multiple thrombi of portal veins. Multiple thrombi of pulmonary arteries. Multiple infarcts of the lungs. General lymphatic hyperplasia.

Histological diagnosis.—Necrosis, congestion, and hemorrhage of the liver. Thrombosis of portal veins and pulmonary arteries. Focal edema and congestion of the lungs with slight leucocytic exudate. Acute endothelial hyperplasia of the lymph nodes. Congestion of the spleen. Acute interstitial (non-suppurative) nephritis. Tests of liver and kidneys for amyloid changes were negative.

HORSE 59.

Clinical notes.—Diphtheria service. Sound active horse about 10 years old. After eight months of treatment was taken suddenly while in the paddock with difficulty of breathing and evident distress. Respiration became very rapid and labored, and at the same time there was very marked spasmodic tenesmus, but no dung was passed. Pulse rate about 90. The horse became weaker as the above condition continued, and finally was unable to stand, and when down struggled violently, so that it became necessary to kill him about five hours after the onset of the symptoms. The maximum antitoxic strength of the serum was 300 units. The last bleeding occurred about 10 days prior to death.

Autopsy notes, anatomical diagnosis.—Thrombosis of portal veins. Focal hemorrhages of liver and spleen.

Histological diagnosis.—Granular degeneration and focal hemorrhages of liver. Leucocytosis.

HORSE 57.

Clinical notes.—Diphtheria service. Sound horse 10 years old. This animal had lived under the same conditions as the other horses in the service for 10 months before the treatment was begun. The toxin injections were pushed with considerable vigor, so that during the four months of treatment he received 14 injections, and had an average of nearly 3.9 days of temperature over 101° F. for each injection given,

which is the maximum for any of the horses in this series. The animal appeared to stand them satisfactorily, and gave a serum of 300 units per c.c. at the second bleeding. Immediately after the third withdrawal of eight liters of blood the animal suddenly fell to the floor in a state of collapse. Nothing was thought of this, as it is not an uncommon occurrence when larger amounts of blood are withdrawn. The horse did not attempt to rise later on, and all efforts to stimulate the animal with strychnin, etc., and to support him in slings, were futile. That night and the following day he struggled violently, and could not be quieted with full doses of morphine or chloral. The next night, and also part of the following day, he rested comfortably. Later he again became violent, and as the temperature rose to 106, and as it seemed impossible to control him, he was killed in the evening. His head and shoulders were badly bruised, and as the autopsy was not performed until the next morning, the tissues and organs showed a well-developed gas bacillus infection at that time.

Autopsy notes, anatomical diagnosis.—Acute endarteritis and thrombus formation in the main trunk of the pulmonary artery. Thrombosis of intrapulmonary branch of left pulmonary artery. Atelectasis of portion of left lung. Hemorrhagic infarct of left lung. Cloudy swelling of the myocardium. Fatty transformation of the liver with evidences of gas bacillus infection. No hemorrhage or other evidence of antemortem necrosis was apparent.

Histology.—Complete postmortem decomposition with gas blebs in the liver and lungs. Tests for amyloid changes in all organs were negative.

HORSE 43.

Clinical notes.—Diphtheria service. Sound horse about 10 years old. Remained well and active during treatment for two years and two months. During the last few months of life was subject to abscess formation at sites of injection. Its serum showed 500 units per c.c. after three months of treatment, and varied from that point to 700 units during the next year, after which time it varied from 300 to 500 units, until three months before death it dropped suddenly to below 200 units. Death occurred in the manner already described.

Autopsy notes, anatomical diagnosis.—Extensive necrosis of the liver with multiple hemorrhages. (See Fig. 1.) Thrombosis of small branch of the portal vein in the right lobe. Rupture of encapsulated hemorrhages in the peritoneum. Hemoperitoneum. Thrombosis of the splenic veins. Hemorrhage and edema of gastrohepatic lymph nodes. Chronic peritonitis.

Histological diagnosis.—Necrosis and amyloid degeneration of the liver, with moderate regeneration. Chronic hemorrhagic cysts of the liver. Organizing thrombi of portal and splenic veins. Amyloid degeneration of Malpighian bodies of spleen. Proliferation of intima of splenic arteries. Hemorrhage and endothelial hyperplasia of gastrohepatic lymph nodes.

HORSE 50.

Clinical notes.—Diphtheria service. Horse of uncertain age, of good condition on entering. Gradually became almost helpless through stiffness of joints and blindness. Had frequent abscess formations at sites of injections. Antitoxic strength of serum never over 300 units per c.c., and usually from 200 to 250 units. Was found dead one morning, and stall showed evidences of continued struggling. The front of the stall was covered with frothy mucus. The coughing-up of this material has never been observed in the horses dying from rupture of the liver.

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Autopsy notes, anatomical diagnosis.—Necrosis and hemorrhage of left lobe of the liver. Congestion of lungs and the right lobe of the liver. General lymphatic hyperplasia. Abscess of subcutaneous tissues of back. Cloudy swelling of heart and kidneys. Acute endarteritis of left renal artery. Thickening of intima of splenic artery. Focal lesions of adrenals. Chronic peritonitis.

Histological diagnosis.—Congestion, edema, hemorrhage, amyloid degeneration, and necrosis of the liver. Chronic interstitial myocarditis. Subpericardial hemorrhage with hyalin degeneration of muscle fibers, and leucocytic infiltration. Edema and congestion of lungs with hemorrhages. Edema and hemorrhages of lymph nodes. Amyloid transformation of Malpighian bodies of the spleen. Localized endarteritis and mesarteritis of left renal artery.

HORSE 56.

Clinical notes.—Diphtheria service. Horse about nine years old on entering. Developed stiffness of joints and became badly crippled. Antitoxic strength of its serum was rarely up to 300 units per c.c., and usually showed but 200 units. After a year and five months of treatment the animal was killed.

Autopsy notes.—Macroscopically and microscopically the liver, spleen, kidney, pancreas, adrenals, lymph nodes, lungs, and heart muscle appeared to be normal.

Table 1 presents a summary of the findings relating to necrosis of the liver and thrombosis in 12 horses which have presented such changes, and also gives those of one horse in whom all the organs were normal, although it had been subjected to active treatment with diphtheria toxin for a period of a year and five months. The other changes found in the tissues of these 12 horses varied to some extent, but such changes did not differ greatly from those to be found in the tissues of fatal cases of diphtheria in man, such as endothelial hyperplasia of lymph nodes, and cloudy swelling of kidney epithelium and heart muscle, etc.

One or two unusual findings were, however, observed. In Horses 43 and 50, belonging to the diphtheria service, amyloid changes were observed in both liver and spleen, and especially in the latter, but were not present in the kidney, heart muscle, or lymph nodes. These animals had developed an unusual number of subcutaneous abscesses at the sites of the toxin injections during the later months of their lives.

In the liver in tetanus horse 21 around the margin of a subcapsular hematoma, and in the necrotic liver of diphtheria horse 43 about the larger portal spaces were found evidences of attempted regeneration in the way of newly formed false bile ducts, and, especially in the latter horse, of columns of newly formed liver cells. In the ne

crotic livers of the other horses there were no evidences of repair. Also in the tetanus horse 29 there were evidences of beginning interstitial nephritis, of the acute non-suppurative type found in diphtheria of man, accompanying widespread disintegration of the epithelium of convoluted tubules.

A study of Table 1 will show that horses presenting necrosis of the liver did not, in all cases, show thrombosis in any organ, nor in animals presenting both conditions was the thrombosis in the hepatic veins always as extensive in its distribution as the necrosis. On the other hand, two diphtheria horses presented thrombosis without evidences of liver necrosis. Unless, therefore, the thrombosis in some instances is secondary and dependent upon the necrosis, and in other cases upon other conditions, we cannot attach much significance to their fairly close association.

That the necrosis is not usually of sudden or rapid development is shown by the fact that the livers of two horses, tetanus horse 21 and diphtheria horse 43, presented encapsulated hematomas of various sizes scattered throughout the liver substance. The thickness of the capsules and the character of their contents indicated their formation at least several months prior to death, which in turn demonstrated the still earlier existence of extensive necrosis.

With the object of ascertaining whether there existed any common factor in the clinical histories of these horses which might assist in arriving at either the etiology of the liver necrosis or the thrombosis, or of both, the individual factors in the history of each horse which has died since these conditions were noted, have been worked out and are compared in Tables 1, 2, 3, and 4.

It is evident from the fact that some horses in the diphtheria, tetanus, streptococcus, and dysentery services presented diffuse necrosis of the liver, that this condition is not due to any special pathogenic property of any one of the organisms used in the immunizations. If, however, it is the result of the activity of some bacterial property, it is not necessary to suppose that the agent in all cases belongs to either the soluble toxins or the so-called bacterial endotoxins, for the reason that the tetanus horses received only toxin which had been filtered through Pasteur-Chamberlain filters, and consequently no bacterial bodies were injected; and, on the other

hand, the streptococcus horses which received large amounts of dead cultures were equally affected.

It hardly seems probable that any of the constituents of the various media used in the preparation of the toxins and toxic products, such as the meat extractives, peptone, etc., could be the causative agent of the liver necrosis, for the reason that the two horses of the streptococcus service (37 and 38) received only about 500 c.c. of broth containing such materials during a period of about one and three-quarters years, the later other injections consisting of suspensions of bacteria or their bodies in physiological salt solution. (See Table 2.)

Neither can any effect produced by the repeated abstraction of blood from such horses be the main cause of the liver necrosis, for, as is seen in Table 2, Horse 36 in the dysentery service was bled only one liter on a single occasion, and the streptococcus horses 37 and 38 had bled eight liters, abstracted on only six and seven occasions respectively during a period of a year and three-quarters. On the other hand, Horse 56 of the diphtheria service was bled eight liters on 18 occasions during a period of a year and a half, and at death its liver was absolutely normal.

TABLE 2.
SHOWING RELATION OF THE MATERIALS INJECTED AND OF REPEATED BLEEDINGS TO LIVER NECROSIS.

Horse	Toxic Broth Injected	Autolyzed or Dead Bacterial Suspensions	Amount Carbolic Acid Injected	Number of Bleedings (8 Liters Each)	Condition at Death	Extent of Necrosis
11.....	42 liters		210 ± c.c.	21	Poor	Extensive
17.....	59 "		295 ±	25	Good	"
28.....	38 "		190 ±	20	Poor	"
36.....		800 c.c.	2½ ±	0	"	"
37.....		4 liters	17 ±	6	Good	"
38.....		4½ "	19 ±	7	Poor	"
21.....	63 liters		165 ±	20	Good	"
29.....	78 "		235 ±	35	"	"
43.....	77 "		385 ±	25	"	"
50.....	50 "		250 ±	15	Poor	Left lobe
56.....	33 "		165 ±	18	"	No necrosis

It is likewise possible to exclude the antiseptics used in the preservation of the bacterial toxic agents as an important factor in the production of liver necrosis, although nearly all the toxic materials administered contained from 0.3 to 0.5 per cent of either carbolic acid or trikresol. A study of Table 2 will show that the actual amounts of these antiseptics received by the different horses varied

from about 2.5 c.c. during 11 months in the case of dysentery horse 36, and about 17 and 19 c.c. in the streptococcus horses 37 and 38 respectively, to 165 and 235 c.c. in the tetanus horses 21 and 29 respectively, and from 190 to 385 c.c. in the various diphtheria horses presenting necrosis of the liver. In other words, the horses receiving the larger amounts of antiseptics lived the longer periods.

Nor could we explain the necrosis of the liver by any general disturbance of metabolism due to the injections of the bacterial toxic agents, for some of the animals which showed the most diffuse necrosis were to all outward appearances and according to their weight in excellent physical condition, as is indicated in Table 2.

There is also no apparent reason for believing that the conditions under which such horses are kept in antitoxin stables is the main factor in the causation of the necrosis. Diphtheria horse 56 was kept under the same conditions as the others for a period of a year and a half, and at autopsy the liver was normal.

A careful study of the temperature charts and a knowledge of the manner in which the horses withstood the injections will, perhaps, give as likely an explanation of the causation of the necrosis as can be found. In Table 3 will be found the number of days during the treatment of each horse, in which the temperature stood at the various points from 101° to 105°+ Fahrenheit. It will be seen that a fair degree of uniformity exists in this number of days of fever in the animals which had necrosis of the liver. These increases of tem-

TABLE 3.
SHOWING RELATION OF REACTIONS DUE TO TOXINS INJECTED TO LIVER NECROSIS.

HORSE	DISEASE	DURATION OF TREATMENT		NUMBER INJECTIONS	DAYS OF TEMP. OVER 101° FAHR.					AVER. DAYS OF TEMPERATURE FOR EACH INJECTION
					101 to 102	102 to 103	103 to 104	104 to 105	Total	
11.....	Diphtheria	1	11	75	95	73	14	1	183	2.4
17.....	"	2	1	77	85	75	22	2	184	2.4
28.....	"	2		69	92	74	38	9	213	3.1
36.....	Dysentery		11	46	86	62	14	4	166	3.6
37.....	Streptococcus	1	9	54	76	44	19	2	141	2.6
38.....	"	1	9½	60	98	48	5	1	152	2.5
21.....	Tetanus	3	3	126	95	69	9	1	174	1.4
29.....	"	3	7	138	135	53	8	2	198	1.4
43.....	Diphtheria	2	2	77	92	65	31	3	191	2.2
50.....	"	1	7	64	79	39	20	0	138	2.1
56*	"	1	5	59	63	57	34	2	146	2.4

*No liver necrosis in this horse.

perature cannot be accepted, however, as an exact index of the character of the reaction, as some horses commonly have considerable fever without any other manifestation of serious disturbance after toxin injections. However, the variations in the temperature curves are due largely to the type of immunization. Thus the dysentery horse had apparently fewer days of temperature than those used in the diphtheria work, but he lived under the treatment only about one-half as long as the latter. It is well known that horses are particularly susceptible to injections of cultures of true or paradyentery bacilli; and this horse withstood the injections very poorly. On the other hand, the horses treated with injections of even large doses of tetanus toxin show only slight disturbances, and in harmony with this is the long duration of the period of treatment in Horses 21 and 29, each of which lived considerably over three years. The two streptococcus horses withstood the injections about as well as the diphtheria animals. Each lived approximately a year and three-quarters, and that was nearly the average life, a year and 11 months, of the five diphtheria horses.

As the horses used in each service lived approximately the same length of time under their respective treatments as, and as the animals receiving the injections of the toxins causing the milder reactions lived for considerably longer periods than, did those receiving toxins producing the most violent reactions, and as we have excluded other possible factors, we are led to the conclusion that the liver necrosis is, without much doubt, mainly the effect of the administration of repeated doses of the bacterial products.

That some of the other factors which appear to be excluded by our findings may be contributory to the main causative agent in some instances cannot be denied.

A possible relationship between the effect of the toxic products of these bacteria on the liver cells and the production of antitoxic or other anti-bodies is not capable of either positive or negative determination from the available data.

In the diphtheria and tetanus horses under consideration no relationship can be made out between the course of the necrotic changes in the liver and the well-known progressive decrease in the antitoxic strength of the sera of some horses after prolonged treat-

ment. Thus diphtheria horse 50, which had necrosis of the liver, gave an antitoxin of from 300 to 350 units per cubic centimeter in only two early bleedings out of a total of 15, the others producing sera of from 200 to 250 units. On the other hand Horse 56, which showed a normal liver when killed after an immunization life of one year and five months, gave almost identically the same grades of serum in 17 bleedings. That is, the early bleedings gave sera of 400 units, and those subsequently from 300 down to 200 units, which was the strength at the time the animal was killed. These findings would seem to indicate that the customary decrease in antitoxic power, which occurs in the sera of many horses as their immunization proceeds after the first few months, is not exclusively due to a progressive liver necrosis. However, the amount of data obtained does not permit us to draw any conclusions concerning the possible relation of the liver to antitoxin production. The bleedings are too infrequent, and the continuation of the antitoxic power of the serum is probably dependent upon so many factors that the absence of liver necrosis in one horse whose serum had shown the usual progressive loss of power, is entirely insufficient evidence to warrant the exclusion of the liver as a factor in antitoxin production.

Brunton and Bokenham¹ have endeavored to show that the liver is capable of producing diphtheria antitoxin by the action of toxin passed through the portal venous system. While it might be questioned whether they have clearly proven the formation of true antitoxin, they undoubtedly showed that the liver cells have an affinity for pure diphtheria toxin, and are capable of at least greatly reducing the poisonous power of the crude product.

A similar affinity of the liver cells of some invertebrates for tetanus toxin has been claimed by Metchnikoff.² He states that in scorpions injected with tetanus toxin the liver is the only organ which absorbs and retains the toxin.

If the liver is capable of absorbing large amounts of the toxic bacterial products, and if their direct action on it is the means of producing liver necrosis, then in most horses treated with such agents the liver is very probably capable of disposing of large amounts of these products without injury; for the liver necrosis is not present

¹ *Jour. Path. and Bact.*, 1904, 10, p. 50.

² *L'Immunité*, etc., Paris, 1901, p. 343.

in many horses, at least, which have come to autopsy after injections of considerable amounts of diphtheria toxin distributed over several months. (See history of Horse 56.)

In view of recent work on autolysis of liver tissues *in vitro*, it may not be out of place to consider the possibility that this late necrosis is due to alterations or disturbances of those factors which prevent the action of the autolytic ferments normally present in the liver.

Baer and Loeb¹ have shown that weak alkalis retard and weak acids stimulate the autolytic ferments in the liver, and that the elements in normal serum which strongly retard or prevent autolysis are the serum albumen and the fibrinogen, while the serum globulin has the opposite effect, namely, a strong stimulating action on the autolytic ferment.

Hiss and Atkinson,² Butjagin,³ and others claim that an increase in the globulin content of the blood occurs during active immunization, although this is disputed by Müller.⁴

Butjagin also demonstrated a gradual decline in the alkalinity of the blood during the later part of the period of immunization.

In view of the work of Baer and Loeb, the occurrence of these or other similar blood changes may have some relation to the development of the liver necrosis or autolysis.

THROMBOSIS.

Of the horses upon which careful autopsies were performed and which presented necroses of the liver, extensive coexisting thrombosis of the veins of that organ was found in one (Horse 21), and to a lesser extent in two others (29 and 43). In the horses treated with streptococcal and dysenteric bacterial products, no careful searches of the entire substance of their livers with this point in view were made.

In one diphtheria (43) and in one tetanus (21) horse the veins of the spleen were likewise the seat of thrombosis to a greater or less extent. The other tetanus horse (29) had extensive thrombosis of the pulmonary arteries and infarction of the lungs.

As has been stated, two diphtheria horses had thrombosis occur-

¹ *Arch. f. exper. Path. u. Phar.*, 1905, 53, p. 1.

² *Jour. Exper. Med.*, 1900, 5, p. 47.

³ *Hyg. Rund.*, 1902, 12, p. 1193.

⁴ *Beiträge z. chem. Physiol. u. Path.*, 1905, 6, p. 454.

ring without necrotic changes in the liver. In one (59) the thrombosis was confined to three large branches of the portal vein in the liver, while in the other (57) the thrombosis was present in the main branch of the pulmonary artery and in the intrapulmonary branch of the left pulmonary artery. In the latter horse the clinical manifestations arose immediately after a withdrawal of the usual eight liters of blood, and may have been due to the formation of a blood clot at the site of this operation and the detachment and passage of the clot into the venous circulation. The thrombus in the main pulmonary artery was in the form of a pedunculated vegetation about the size of a walnut. The horse apparently suffered considerable thoracic pain, and was so weak as to be unable to stand even with the assistance of slings.

The other horse (59) with localized thrombosis of the portal veins gave indications of great pain in the abdominal cavity, with tenesmus, and also complete prostration and excessively rapid respiration and a high pulse rate. After the continuation of these symptoms for about five hours the animal was killed, and the autopsy performed immediately.

If the conditions operating to produce the thrombosis in this case are the same as in those where the thrombosis coexisted with liver necrosis, it is remarkable that it should have occurred as early as the eighth month of the immunization period, and that the clinical symptoms should be so much more severe than in those cases where thrombosis occurred later and more extensively.

In looking for some factor or condition operative during the period of treatment which might be considered as of etiological significance for the thrombosis, we are not afforded as much data as we would like, owing to the failure to examine and note particularly the presence or absence of thrombi in the first few animals which died from rupture of the liver with hemorrhage. Thus we have no definite information on this point in three diphtheria, one dysentery, and two streptococcus horses.

From the data tabulated in Table 4 it can be seen that by excluding the horses with early thrombosis the treatment given and its effects on the three remaining horses are quite similar. The only exception is to be found in a considerable difference in the amounts

TABLE 4.
RELATION OF THE TREATMENT OF HORSES TO THE DEVELOPMENT OF THROMBOSIS.

No.	Diseases	Immunization Period	Liters of Broth Injected	Amount of Carbolic Acid Injected	Days of Tem. over 101°	Number of Bleedings of 8 Liters Each
59.....	Diphtheria	8 mos.	16½	82 ± c.c.	91	7
57.....	"	4 "	5½	27 ±	54	43
21.....	Tetanus	3 yrs. 3 "	63	105 ±	174	29
29.....	"	3 " 7 "	78	235 ±	198	35
43.....	Diphtheria	2 " 2 "	77	385 ±	191	25

of carbolic acid or trikresol which the horses received with the toxins injected. We are of the opinion that these antiseptics played no essential part in the production of the conditions favoring thrombosis.

We cannot, however, readily exclude in this way a possible influence favoring thrombosis by the injection of those elements which make up the broth used for toxin production, such as peptone meat extractions, etc. Likewise we cannot exclude the effects of the repeated abstraction of eight liters of blood every three or four weeks. We are unable, therefore, by any process of exclusion to form any definite opinion of the part played in the thrombosis by the pathogenic activity of the bacterial toxins injected into these horses.

If, however, we ascribe to the early thromboses the same etiological conditions as bring about the late thromboses, then we can find no common factor in the treatment of our horses which might have been operative in the determination of this condition.

There are, however, some noteworthy features in the relation of thrombosis to the liver necrosis in some of the horses.

Thus in Horse 50, which was the only animal with necrosis of the liver which was thoroughly examined for thrombi and none found, the necrosis and hemorrhage of the liver was not general but localized almost exclusively in the left lobe, and was evidently not so far advanced as in the other animals. As the autopsy also revealed myocarditis with small subpericardial hemorrhages, and amyloid degeneration of the liver and spleen, local endarteritis, edema, and congestion of the lungs with small hemorrhages, it is not unlikely that the animal did not die from the effects of the liver necrosis, especially as there was no rupture of that organ. It is not improbable, therefore, that thrombosis would also have occurred in this horse if he had lived longer.

In Horse 29 the portal veins in which thrombi were found corresponded closely with the areas of necrosis in the liver, and it was noted that the thrombosis seemed to proceed from the smaller veins outward into the larger branches, ending there in large, round-ended clots.

In Horse 43 the extensively necrotic liver showed thrombosis only in one small portion of the right lobe, but considerable thrombosis occurred in the spleen.

Thus, taking the findings as a whole, it can be stated that while all necrotic areas in the liver did not have coexisting thrombosis, there were no thrombi in the portal veins other than in the necrotic portions, except in the two horses already described which died with early thrombosis without necrosis. It is possible, therefore, that in some animals, at least, the portal thrombosis may be caused by lesions of the vessel walls secondary to the liver necrosis.

It is the opinion of Smith¹ that the pathological effects of the periodic losses of blood in antitoxin horses lead in certain cases to serious derangements and death. He shows that in many horses which have been bled five liters every three or four weeks for periods of from nine months to over four years, the red blood corpuscles have a reduced osmotic tension. He believes that this condition is due to the repeated bleedings and not to the toxin injections.

In Pettit and Girard's case of necrosis of the spleen in an anti-plague horse, the authors express the qualified opinion that the necrosis was not due to the periodic bleedings.

In so far as necrosis of the liver is concerned, our data do not warrant the conclusion that the periodic bleedings have any special etiological relation to it. If, therefore, the lowered osmotic tension is due to the repeated losses of blood, it is not probable that such lowered tension is in any way responsible for the liver necrosis. Whether it is or is not a factor in the causation of the thrombosis we are not prepared to state.

Butjagin has shown the presence of this phenomenon and also the existence of such alterations in the blood serum as an increase in the specific gravity, changes in albumen and globulin content, electrical conductivity, alkalinity, and refractive index during the immunizing process.

¹ *Jour. Med. Res.*, 1904, 12, p. 385

Similar work on one or more of these aspects of the subject has been reported by Müller¹ and others.

What relation exists between these alterations in the morphological, chemical, and physical conditions of the blood constituents and the pathological conditions noted by us, remains to be solved.

SUMMARY.

In a series of 12 horses, actively immunized with the products of certain pathogenic microorganisms, autopsies revealed widespread liver necrosis in nine, and localized liver necrosis in one.

Thrombosis of the portal veins was present in three, of the splenic vein in two, and of branches of the pulmonary artery in one, of the nine horses showing widespread necrosis. One horse had thrombosis of branches of the portal veins and another of the pulmonary artery without liver necrosis. Six horses were not carefully examined for thrombosis.

The evidence obtained tends to exclude the materials such as peptone, beef extractives, and antiseptics, such as carbolic acid, injected with the toxins, and also the repeated bleedings practiced, from any important rôle in the production of the liver necrosis. On the other hand, the evidence leads to the conclusion that the toxic bacterial products injected are directly or indirectly responsible for the production of this pathological condition.

The evidence is not sufficient to determine which, if any, of these factors is responsible for the occurrence of the venous thrombosis. A possible etiological action by the antiseptics injected is excluded. In some cases the portal thrombosis may result from the liver necrosis.

NOTE.—Since this paper went to press another horse (No. 60) has died after a year and four months of treatment. During the first four months of this period it received diphtheria toxin, and after that until death small doses of tetanus toxin. The horse had a very slightly varying temperature of from 104° to 106° during the two weeks prior to death, and gradually became weaker during the last few days of life, and died during the night. Just prior to the onset of the high temperature an edematous condition of the genital sheath developed, followed by a tremendous increase in the size of the penis, apparently due to venous stasis. Later the use of the left hind leg became impaired.

At autopsy thrombosis of the left iliac vein, the inferior vena cava, the veins of the tissues about the penis, the splenic vein outside the spleen, the portal vein, and some of its branches in the liver, and of some of the pulmonary arteries was found. There was no evidence of necrosis in the liver. There was a general hyperplasia of the lymph nodes.

¹ *Loc. cit.*